

Restraining the non-small cell lung cancer (NSCLC) tumor by using MART 10, a vitamin D analog and E-Cadherin

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Abstract

NSCLC is one of the most common types of cancer. Previous studies have reported that MART 10 has been affecting anaplastic thyroid cancer, indicating a decrease in the tumor after the injection of MART 10. Since previous studies rarely investigate the effect of MART 10 within the NSCLC, this study investigates the effect of MART 10 in the NSCLC, both in vivo and in vitro methods. The study will use A549 cell lines, with negative control PBS and positive control EGFR inhibitors or increasing MART 10—various methods, both in vitro, such as MTT, western blot, and wound healing assay. In vivo, such as A549 mice xenografts, the paper investigates whether or not MART 10 can restrain the development of NSCLC tumors.

Keywords: NSCLC, MART 10, Vitamin D analog, A549

1. Introduction

Lung cancer is one of the most common types of cancer [1]. It is estimated that every year, there are around 228,150 new cases globally [2]. Research has shown that the 5-year survival rate for all people is 22%, which is quite a low percentage. The mortality rate of this disease is very high [3]. The major risk factor for non-small cell lung cancer includes radon exposure, genetics, air pollution, radiation, and smoking. Social class can also affect the chances of getting lung cancer, in poor socioeconomic conditions (lower class) there will be a higher chance of suffering from these sicknesses. But some people can not afford the expensive treatment that is now available. Therefore, conducting this experiment will hopefully find results that could help with the current medication for non-small lung cancer.

The treatment currently for stage 0 non-small cell lung cancer includes PDT (photodynamic therapy), laser therapy, and brachytherapy. Stage 1 includes SBRT (stereotactic body radiation therapy) and RFA (radiofrequency ablation). For stage 2, there are drugs such as EGFR inhibitors that can be taken. For stage 3 and above, it is recommended to get chemotherapy, surgery, and radiation therapy [4,5]. But recent discoveries had shown that E-cadherin, which is a type of protein, along with a vitamin D analog, MART 10 has been making a good effect on anaplastic thyroid cancer [6]. Since MART 10 can repress the ATC cell migration and its invasion through the reversal of the EMT through cadherins. This observation led to the question of whether MART 10 and E cadherin also be able to restrain non-small cell lung cancer.

MART 10 is a new brand of vitamin D analog. It has been shown that it's an effective drug to inhibit HNSCC growth [7]. This finding shows that MART 10 can be used as an effective drug in many ways, other than its uses in anaplastic thyroid cancer. It has promising results for NSCLC [8]. One of the current treatments for non-small cell lung cancer is EGFR inhibitors. EGFR is a protein that is found on the surface of some cells. It is found at really high levels in cancer cells, and its activation seems to be important for tumor growth. Two of the EGFR inhibitor includes tyrosine kinase inhibitors, which stops the activity of the EGFR. The other one is the monoclonal antibodies, and which prevent the binding of EGFR to epidermal growth factor, therefore stopping cell division and survival.

2. Hypothesis

I predict treatment with increasing amounts and for various durations with MART10 will increase E-cadherin and decrease N cadherin and will be able to restrain the proliferation of the A549 non-small cell lung cancer cell line. After treatment with MART10, measure cell proliferation by MTT assay, measure decrease in tumor size using a mouse xenograft system, measure cell migration by wound healing assay, and measure ratio E/ N cadherin by western blot. The positive control is EGFR inhibitors, the negative control is PBS.

Therefore, due to the limited information regarding MART 10 and non-small cell lung cancer, this paper will be hypothesizing that MART 10 does have an actual effect on the decreasing of the tumors within non-small cell lung cancer.

3. Materials and Method

3.1. Cell Line

This experiment will use 1 known cell line, A549(non-small cell lung cancer cell line) that exhibits a high and low expression of MART 10 respectively.

In Vitro Cell Culture

A549 will be cultured with 10% fetal calf serum, 100ug/ml streptomycin, and 100 U/ml penicillin, kept at 37 C in a humidified environment with 10% CO₂ and 90% O₂.

3.2. Western Blot Analysis

After three days of treatment with MART 10, PBS, and EGFR inhibitors, cells will be collected and washed with PBS, then lysed with RIPA lysis buffer. The antibodies used in this experiment are mouse monoclonal primary antibodies against E cadherin and HRP-conjugated anti-mouse secondary antibodies. After washing in TBST, blots were detected by using the ECL reagents. The expression of targeted proteins relative to tubulin (as the loading control) was calculated. The experiment was performed in triplicates.

3.3. MTT Assay

Cell viability will be determined by MTT cytotoxicity assay. Cells will be seeded on 96-well culture plates at 5×10^3 cells per well in a serum-free media, such as DMEM, which contains 10% FCS. After 24 hours of incubation, cells will be treated with EGFR inhibitors (erlotinib), no drug, or MART 10. Those treated with MART 10 will receive 0.1, 1, 10, 100, or 200 ug/ml of MART 10, respectively. MTT assays will be performed after 24, and 48, 72 hours of MART 10 treatment. The experiment was performed in triplicates.

3.4. Animal Experiment

A549 cell lines will first be transplanted subcutaneously to the tails of 30 nude mice at 10×10^5 cells per xenograft. A week later, the respective amounts of PBS, EGFR inhibitors (erlotinib), or MART 10 (0.1, 1, 10, 100 or 200 ug/day) will be administered intraperitoneally to A549-transplanted mice every 2 hours for a consecutive 10 days. The mice will be divided into 3 groups: MART 10, negative control with PBS injection, and positive control with EGFR inhibitors(erlotinib) injection. After the 10-day cycle, tumors will be excised, immersed in 10% buffered formalin of neutral pH, dehydrated, and embedded in paraffin, for eventual operations of immunohistochemistry and hematoxylin-eosin staining. The experiment was performed in triplicates.

3.5. Wound Healing Assay

A549 cell lines will be stored in a plate. There will be

two different groups, one will be injected with MART 10 and the other one will be just A549 cell lines. Both will be scratched with the tip of a pipette. After 8 hours, the increase of the gap within the cell will be measured. The experiment was performed in triplicates.

3.6. Statistical Analysis

The statistical significance of all numerical data collected from Western Blot, MTT, Wound healing, and animal experiments will be analyzed using the T-Test. The level of significance will be set at $p < 0.05$.

4. Results

Combined result 1: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing increased. This result fully supports my hypothesis.

Combined result 2: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 3: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor increased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 4: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 5: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 6: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor increased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 7: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor increased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 8: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing

decreased. This result partially supports my hypothesis.

Combined result 9: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 10: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor increased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 11: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 12: This result shows that the ratio of E/N cadherin was increased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor increased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 13: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was decreased through MTT assay. The weight of the xenograft tumor increased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 14: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor decreased, and the wound healing decreased. This result partially supports my hypothesis.

Combined result 15: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor increased, and the wound healing increased. This result partially supports my hypothesis.

Combined result 16: This result shows that the ratio of E/N cadherin was decreased by western blot. The proliferation of cell was increased through MTT assay. The weight of the xenograft tumor increased, and the wound healing decreased. This result does not support my hypothesis (Table 1).

Table 1: Combination of Possible Results (CR)

Possible Observations	CR 1	CR 2	CR 3	CR 4	CR 5	CR 6	CR 7	CR 8	CR 9	CR 10	CR 11	CR 12	CR 13	CR 14	CR 15	CR 16
Ratio of E/N cadherin increased by WB?	+	+	+	+	-	+	+	+	-	-	-	+	-	-	-	-
Cell proliferation decreased by MTT?	+	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-
Weight of xenograft tumor decreased?	+	+	-	+	+	-	-	+	+	-	+	-	-	+	-	-
Wound healing increased?	+	-	+	+	+	+	-	-	-	+	+	-	-	-	+	-
Support hypothesis?	YES	PT	PT	PT	PT	PT	PT	PT	PT	PT	PT	PT	PT	PT	PT	NO

Note. + means similar results as the EGFR inhibitors (erlotinib) and different results as PBS

- means similar results as PBS and different results as the EGFR inhibitors (erlotinib)

(PT = partially)

5. Discussion

Combined result 1 fully supports my hypothesis. The general result was similar to the EGFR inhibitors (erlotinib) and different from the results of PBS. There was never any previous experiment on this particular topic before, but it should work. Since there are research that had shown that the increase of E cadherin can restrain NSCLC. If it works, then it means that the injection of MART 10 caused the reverse of E cadherin, which will activate cell-cell adhesion, and therefore would stop metastasis. MART 10 had made a similar result to the anaplastic thyroid cancer before. But it's also important to note that, there might be errors with concentration and duration. For example, the timing might be inaccurate, or the concentration might be too much or too little. In this result, it seems reasonable that if there was higher concentration of MART 10, the duration of the reaction would probably take less time. Because it can quickly activate everything, and it will show the adhesion quickly which will stop further spreading.

Combined result 2 to 15 all partially supports my hypothesis. Some of the results were similar to EGFR inhibitors (erlotinib) and some were similar to PBS. According to previous (related) research, these results are highly likely to happen as well. Because it is possible that there might be some errors within the experiment. It's very hard to get the precise result, so there might be 1 or 2 experiments not working. But if these experiments just happen to not be working then it shows that MART 10 restrain NSCLC only in specific ways. Results that had shown to be working with the xenograft models, shows that the in vivo process works better than the vitro process. This might happen due to the fact that the surroundings of the mouse might be more suitable for MART 10 to work in, since it's an entire body. There might specific details we might not be able to get in in vitro processes.

Combined result 16 does not support my hypothesis at all. All of the results were similar to the PBS' result, and different from EGFR inhibitors(erlotinib). According to previous research, this result is unlikely. Because of all the effects that MART 10 seems to be making is beneficial in previous related topics. Such as the anaplastic thyroid cancer. But of course, due to the fact that there might be errors, this is also a possibility. But if the experiments were carried out properly, and the result still looks similar to this, then it means that the E cadherins have not been reversed. Therefore, it did not stop the metastasis.

6. Conclusion

In general, this study investigates the effect of MART 10 restraining the development of the NSCLC through in vitro methods, such as MTT, western blotting, wound healing assay, as well as in vivo method that studies Xenografted Mice. The results of this study will indicate whether or not MART 10 does have a positive effect toward the A549 NSCLC cell line. Future studies could either focus on investigating the combinations of MART 10 with various anti-cancer chemotherapy drugs, such as EGFR inhibitors, and examine if any results may be derived.

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